Effects of Carbon Dioxide Enrichment on Leaf Chemistry and Reproduction by Twospotted Spider Mites (Acari: Tetranychidae) on White Clover

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ABSTRACT Plant growth and yield responses to carbon dioxide (CO_2) enrichment are well established. Much less is known of the response of arthropod pests to CO_2 enrichment. Reproductive response of twospotted spider mites (*Tetranychus urticae* Koch) on white clover (*Trifolium repens* L.) to a range of CO_2 concentrations was measured. The CO_2 treatments were applied for 24 h d^{-1} at ≈395, 484, 570, 657, and 748 μLL^{-1} on the 14 d before and 26–27 d after infestation with mites. Eggs, larvae, nymphs, and adult mites were removed from leaves and counted 27–29 d after infestation. Leaf area and weight were measured, and leaves were analyzed to measure structural and nonstructural carbohydrates, N, amino acids and digestibility. Carbon dioxide enrichment caused linear increases in plant growth and foliar nonstructural carbohydrates, but caused linear decreases in foliar N. Carbon dioxide enrichment significantly increased the rate of mite reproduction on both clover clones. Correlations between mite population increase were significantly positive for foliar nonstructural carbohydrates and significantly negative for foliar N. Concentrations of ambient CO_2 expected in the 21st century may increase the risk of mite population damage on some plant species.

KEY WORDS *Trifolium repens, Tetranychus urticae,* white clover, carbon dioxide enrichment, twospotted spider mite

INCREASED ATMOSPHERIC CARBON dioxide (CO_2) increases photosynthesis, growth, and yield of most plant species (Rogers et al. 1983a, Cure and Aycock 1986, Kimball 1986, Bazzaz 1990), and it is widely believed that CO_2-induced changes can also alter nutritive value for herbivores. Insects in some feeding-guilds will likely be affected differently than insects from others, and this subject was recently reviewed (Bezemer and Jones 1998). For example, leaf chewing insects generally consume more foliage of plants grown at elevated than at ambient CO_2 (≈360 μLL^{-1}) but insect growth and development generally has not been affected or has been suppressed (Weste et al. 1987, Akey and Kimball 1989, Lincoln 1993, Bezemer and Jones 1998, Brooks and Whittaker 1998, Buse et al. 1998, Lindroth and Kinney 1998, Williams et al. 1998). Populations of six leaf miner species on oak decreased in elevated CO_2 (Stiling et al. 1999), and a trend for decreased larval size occurred for another leaf miner (*Pegomya nigritarsus* Zetterstedt) on dock (Rumex spp.) (Salt et al. 1995). Also, populations of a spittlebug (*Neophilaenus lineatus* L.) feeding on xylem of *Juncus squarrosus* L. were decreased by elevated CO_2 (Brooks and Whittaker 1999). Populations of phloem-feeding aphids often respond positively to elevated CO_2. Reproduction of *Myzus persicae* (Sulzer) on groundsel (*Senecio vulgaris* L.) and annual blue grass (*Poa annua* L.) was increased by CO_2 enrichment (Bezemer et al. 1998). The same was true for *Aulacorthum solani* (Kalt.) on bean (Awmack et al. 1997) and *Sitobion avenae* (F.) on winter wheat (Awmack et al. 1996). A consistent, although not significant, trend for population increase occurred for *Aphis fabae fabae* Scopoli on cardamine (Salt et al. 1996). However, populations of *Phyllaphis fagi* L. on beech, and *Drepanosiphum platanoidis* (Schrank) and *Periphyllus testundinaceus* (Ferni) on sycamore were not significantly affected by CO_2 enrichment (Docherty et al. 1997). Carbon dioxide enrichment caused a trend for increased reproduction of *Myzus persicae* on Brussels sprouts (*Brassica oleracea* L.) but significantly decreased reproduction of *Brevicoryne brassicae* (L.) on the same species, showing that insect response to elevated CO_2 can depend on both the host plant and aphid species. (Bezemer et al. 1999).

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Only a few reports of CO_2 enrichment on whole-cell feeding arthropods exist, and responses have been mixed. No significant population change occurred for the sweet potato whitefly [*Bemesia tabaci* (Gennadius)] on cotton (Butler et al. 1986) or for western flower thrips [*Frankliniella occidentallis* (Pergande)] on milkweed (Hughes and Bazzaz 1997). Populations of greenhouse whiteflies [*Trialeurodes vaporariorum* (Westward)] on tomato decreased with CO_2 at 1,000 μLL^{-1} (Tripp et al. 1992). We previously measured population growth response of the twospotted spider mite (*Tetranychus urticae* Koch) to ambient and double ambient CO_2 concentrations on an O_3-sensitive (NC-S) and an O_3-resistant (NC-R) clone of white clover (*Trifolium repens* L) (Heagle et al. 1994a). Double-ambient CO_2 decreased foliar N concentrations and stimulated *T. urticae* population growth (Heagle et al. 1994a, Burns et al. 1997). Conversely, double ambient CO_2 decreased reproduction of the "White Eye" strain of *T. urticae* on *Phaseolus vulgaris* L. leaves (Joutei et al. 2000).

The twospotted spider mite is one of the most important plant pests worldwide. It feeds on leaves of over 150 plant species including field, vegetable, and fruit crops (Jeppson et al. 1975). Most research on effects of CO_2 enrichment has involved only two CO_2 concentrations. Our previous study (Heagle et al. 1994a) employed only two CO_2 levels and the only nutritive factors measured were total N, protein N, in vitro dry matter disappearance and neutral detergent fiber (Burns et al. 1997). Dose-response studies to measure effects of a wide range of CO_2 concentrations on plant pests have not been reported. Our objectives were to measure mite population response on *T. repens* to five CO_2 concentrations to allow development of dose-response models, and to determine if correlations exist between mite population response and CO_2-induced changes in nutritive value of white clover leaves (e.g., N, structural and nonstructural carbohydrates, and individual amino acids).

Materials and Methods

General. The experiment was performed in a nonfiltered-air greenhouse from December to February in each of two years. The O_3-sensitive (NC-S) and O_3-resistant (NC-R) clones of white clover were propagated by rooting virus-free stolons in 1-liter pots containing a 2:1:1 mixture of sandy loam soil:sand:Metro Mix 220 (Scotts-Sierra Horticultural Products County, Marysville, OH) in trial 1 and in Metro Mix 220 in trial 2. Details of the selection of NC-S and NC-R and their relative response to elevated O_3 and CO_2 are published (Heagle et al. 1993, 1994b). Plants were watered as needed to prevent wilting and were fertilized at ≈2-wk intervals with 250 ml of a solution containing 1 g of a 5:11:26 (N:P:K) fertilizer per liter of water.

Plants were exposed to CO_2 in 10 cylindrical (1.07 m diameter by 1.20 m tall) continuous-stirred tank reactor chambers (CSTRs) continuously aspirated with charcoal-filtered air (Heck et al. 1978). Ambient greenhouse light was supplemented by a 1,000-W multivapor lamp that supplied ≈270 $\mu mol\ m^{-2}\ s^{-1}$ of photosynthetically active radiation to each chamber from 0600 to 1800 hours EST daily. Temperature was monitored with copper-constantan thermocouples, photosynthetically active radiation was measured with quantum sensors (LI-190S-1, LI-COR, Lincoln, NE), and relative humidity was measured with Vaisala HMP 31UT sensors (Vaisala, Woburn, MA).

We used mites originating from *T. repens* in the field for trial 1 and from *T. repens* in a greenhouse for trial 2. Mite colonies were maintained on NC-S and NC-R in the greenhouse for at least 30 d before each trial. The sequence of events for both trials was as follows: starting at 20 or 22 d after rooting of clover cuttings began, plants were exposed to CO_2 for 14 d before they were infested with adult mites. The CO_2 exposures continued for an additional 26–27 d, and mite populations, plant growth, and plant chemistry were measured.

Exposures. The experimental design was a randomized complete block with two blocks of five CO_2 concentrations as the main plot (CSTRs) treatments and the two clones as split plot treatments with four pots of each clone in each of the 10 CSTRs. Carbon dioxide was obtained from tank CO_2 and was monitored sequentially in each chamber with an infrared CO_2 analyzer. An Ar-500R (Anarad, Santa Barbara, CA) CO_2 analyzer was used in trial 1, and a LI 6252 (LI-COR) was used in trial 2. Details of CO_2 dispensing and monitoring protocols used in this study have been described (Rogers et al. 1983b).

Uniform infestation of clover with mites was accomplished by using a small paintbrush to place one adult female on separate leaves of four plants per clone per CSTRs 14 d after exposures began. In trial 1, 10 leaves per plant were infested and in trial 2, seven leaves per plant were infested. Exposures continued for 26–27 d after infestation allowing time for ≈1.5–2.5 generations (Hummel et al. 1998). To minimize possible effects of chamber differences in microclimate, plants were rotated among the five CSTRs within each block at 7 and 14 d after infestation for trial 1 and at 9 d after infestation for trial 2. Meteorological conditions during exposure for both trials are described in Table 1. Mean 24 h d^{-1} CO_2 concentrations for the 42 d of exposure in trial 1 were 392, 482, 568, 657, and 746 μLL^{-1} and for the 41 d of exposure in trial 2 were 398, 486, 568, 658, and 751 μLL^{-1}.

Response Measures. All leaves were sampled to count eggs, larvae, nymphs, and adults within 3 d after exposures ended. To avoid confounding due to differences in sampling time, the sampling order was one pot per clone per successive CSTR. All leaves were individually brushed with a mite-brushing machine (Leedom Engineering, Twain Harte, CA) that deposited all mite life stages on a glass plate covered with a detergent film. A microscope was used to count each life stage from each plant sample. After leaves were brushed to remove mites, laminar area was measured with an electronic meter (LI-3100, LI-COR) and fresh weights of laminae and petioles were measured.

Table 1. Means and range among the continuous-stirred tank reactor chambers (CSTRs) for temperature, relative humidity, and photosynthetically active radiation (PAR) during exposures of white clover to carbon dioxide

Trial	Dates of exposure	Date of infestation	Dates of population measurements	Temp, °C	% RH	PAR mol m^2d^{-1}
1	4 Jan–14 Feb	18 Jan	15–16 Feb	19.6 (19.5–19.8)	60.2	20.5 (18.8–21.2)
2	22 Dec–31 Jan	5 Jan	1–3 Feb	20.1 (19.7–20.4)	55.3	27.6 (24.5–31.0)

Mean and range for temperature (7 CSTRs), PAR (4 CSTRs), and relative humidity (1 CSTR in trial 1,4 CSTRs in trial 2).

Laminae and petioles were placed in a freezer, and were freeze-dried for analyses to measure factors that may affect nutritive value. Following freeze-drying, the dry weight of each sample was measured, and the tissue ground in a cyclone mill to pass a 1-mm sieve. Samples were returned to a freezer (-16°C) until analyzed. Laboratory determinations, used in calibration of a near-infrared reflectance spectrophotometer to develop prediction equations, were made for total nitrogen (AOAC 1990) using an Auto-Analyzer (Technicon Industries System, Tarrytown, NY) and total fiber fractions. Fiber fractions, consisting of the cell walls (neutral detergent fiber [NDF]), and constituent fractions [acid detergent fiber (ADF)] and acid detergent lignin (72% sulfuric acid), were determined according to Van Soest and Robertson (1980) in an Ankom 200 fiber extractor (Ankom Technologies, Fairport, NY). Cellulose (CELL) was determined by subtracting lignin and ash from ADF. Nonstructural carbohydrates were analyzed by an adaptation (Fisher and Burns 1987) of the method by Smith (Smith 1981) in which monosaccharides (mono), mono+disaccharides (MD), fructans and starch were separated by differential solubility. Starch was determined by digesting to glucose with amyloglucosidase and reading the monomer concentration on a YSI model 27 Industrial analyzer (Yellow Springs Instrument, Yellow Springs, OH). An estimate of ruminant digestibility (in vitro true dry matter disappearance) was determined using a 48-h incubation with ruminal inoculum in a batch fermenter (Ankom Technology, Fairpont, NY) followed by extraction in neutral detergent solution for determination of in vitro true dry matter disappearance. Amino acids in leaf laminae were determined by hydrolyzing samples containing ≈10 mg of protein in 10 ml of 6 M HCl for 20 h at 100°C in a nitrogen digestion block. The hydrolyzed sample was taken to dryness then derivatized with heptafluorobutyryl (HFB) in isopropanol (IB) using an HFB-IB amino acid kit (Alltech, Deerfield, IL) giving the N (O, S)-heptafluorobutyl-isobutyl esters of the amino acids. Amino acid separations and concentrations were determined using a 3800 Varian gas chromatograph with an 8200 auto sampler (Varian, Sugarland, TX). Nitrogen was used as the carrier gas and passed at four psi through a 25 m × 0.53 mm i.d., Heliflex AT-amino acid capillary column. The injector and detector were set at 250 and 275°C, respectively. The column was temperature programmed with the initial temperature set at 80°C for 2 min, increased to 225°C at 5°C min^{-1} and held constant for 4 min, then raised to 250°C at 50°C min^{-1} and held constant for 9 min. Amino acids were not determined for petiole tissue.

Statistical Analyses. Analyses of mite population and leaf growth data were performed using the chamber means for each clone. Data for each trial were analyzed separately using general linear models and results were examined for similarity in treatment responses across trials. Data for the two trials were then analyzed together with both trial and block treated as random factors. Residual plots were examined for nonnormality, outliers, and heterogeneous variances. If the variances for the two trials were significantly different for a variable, the combined analysis was rerun with the observations from each trial weighted by the inverse of the error variance from the data set for that trial. The residual plots from the weighted least squares analysis were then examined to determine whether or not weighting successfully corrected the heterogeneity problem. The Box-Cox Test (Rawlings 1988) was also used to determine whether transformation could be used to correct effectively for heterogeneity or improve normality. All leaf growth data and mite nymph + adult data were analyzed using their original scales. Egg, larvae, and total mite counts were analyzed using weighted least squares. Orthogonal contrast statements were used to obtain the significant polynomial effects for CO_2 and its interaction with trial and clone. The three-way interaction (CO_2 × clone × trial) was never significant and so was not partitioned.

Nutritive quality data in laminae and petioles were analyzed separately using generalized least squares (SAS Institute 1985). The first analysis combined the two trials, with CO_2 concentration and clones as main effects. The second analysis was conducted only on trial 2, and the main effects were CO_2 concentration and clones. The two-way and three-way interactions were generally not significant in either of the analyses. Linear trend analysis ($P \leq 0.05$) was used to determine CO_2 effects (SAS Institute 1985). Because of the smaller number of degrees of freedom, clone main effect differences were determined a priori to be tested at $P \leq 0.10$. Simple linear correlation was used to examine the relationship of mite populations and laminae nutritive value (SAS Institute 1985).

Results

Foliar Nutritive Value. Carbon dioxide enrichment caused linear increases (percentage of dry weight) of all nonstructural carbohydrates in both laminae and petioles, except for monosaccharides in laminae (Table 2). Conversely, percent N decreased linearly with CO_2 enrichment in both laminae and petioles, prob-

Table 2. Nitrogen and nonstructural carbohydrates in clover clones exposed to different concentrations of CO_2 and infested with twospotted spider mites[a]

Leaf portion	CO_2 concn (μLL^{-1}) or clone		Nonstructural carbohydrates (NC), g kg^{-1}[a]					
			Nitrogen	Mono-saccharides	DI-saccharides	Fructans	Starch	Total NC
Laminae	395		45	54	4.7	4.6	65	128
	484		45	55	4.9	4.8	82	147
	570		41	56	5.2	5.0	94	160
	657		40	54	5.5	5.3	116	181
	748		39	57	5.6	5.4	123	191
		Trend ($P \leq 0.05$)	Linear	NS	Linear	Linear	Linear	Linear
	NC-S		44	61	5.2	4.9	79	15.0
	NC-R		39	50	5.2	5.1	113	17.3
		P	0.01	0.01	0.57	0.62	0.01	0.01
Petioles	395		20	84	8.9	3.7	27	124
	484		19	87	9.0	3.9	33	133
	570		18	87	9.1	4.1	34	134
	657		17	95	9.4	4.8	41	150
	748		17	96	9.4	4.8	39	149
		Trend ($P \leq 0.05$)	Linear	Linear	Linear	Linear	Linear	Linear
	NC-S		18	97	9.3	4.2	35	146
	NC-R		18	83	9.0	4.4	34	130
		P	0.75	0.02	0.01	0.71	0.05	0.01

[a] Each value for CO_2 responses is the mean of two clones, two trials and two replicates within years (=8). Each value for clone responses is the mean of five CO_2 concentrations, two trials and two replicates within trials (=20).

ably because of dilution caused by increased concentrations of carbohydrates. Correlations of N with starch and total nonstructural carbohydrates in the laminae were consistently strong and negative ($r = -0.96$ to -0.98, $P \leq 0.001$). Concentrations of N and monosaccharides were significantly less in laminae of NC-R than in NC-S but concentrations of starch were highest in laminae of NC-R (Table 2).

Carbon dioxide enrichment did not significantly ($P \leq 0.10$) affect the concentration of any of the 14 amino acids analyzed in leaf lamina, although the clone effect was significant for several amino acids (Table 3). For example, NC-R contained significantly lower concentrations of aspartic acid, glutamic acid, leucine, lysine, phenylalinine, proline and threonine than NC-S, and concentrations of most other amino acids were also lower in NC-R than in NC-S (Table 3). The clone $\times$ CO_2 interaction was not significant for any amino acid.

Carbon dioxide enrichment did not significantly affect structural carbohydrates (NDF, ADF, and CELL) or in vitro true dry matter disappearance in laminae or petioles. For the clone $\times$ CO_2 treatments combined, mean concentrations (g kg^{-1}) of structural carbohydrate fractions in laminae were as follows: NDF = 162, ADF = 95, and CELL = 79. Comparable concentrations in petioles were as follows: NDF = 292, ADF = 224, and CELL = 203. Concentrations (g kg^{-1}) of in vitro true dry matter disappearance were 961 in laminae and 934 in petioles.

Leaf Growth. Leaf laminae area, leaf laminae weight, and leaf petiole weight generally increased with increased CO_2 concentration (Table 4; Fig. 1). The increased leaf weight was probably caused by increased carbohydrate concentrations. For the trials combined, the CO_2 linear effect was significant for laminae and petiole weight and for laminae area (Table 6). Specific leaf area (SLA = laminae area/laminae weight) increased with increased CO_2 for both clones in trial 2 but no consistent trend for this response occurred in trial 1 (Table 4), and the CO_2 effect on

Table 3. Amino acid concentrations in leaf laminae of clover clones exposed to different concentrations of CO_2 and infested with twospotted spider mites[a]

Clone		Amino acids (g kg^{-1} dry wt basis)[a]							
		Alanine	Aspartic acid	Glutamic acid	Glycine	Isoleucine	Leucine	Lysine	
NC-S		2.24	2.80	2.61	1.59	0.98	2.28	1.25	
NC-R		2.44	2.53	2.36	1.57	0.90	2.13	1.13	
	P	0.08	0.03	0.03	0.72	0.12	0.09	0.01	
		Methionine	Phenylalanine	Proline	Serine	Threonine	Tyrosine	Valine	Total
NC-S		0.41	1.31	1.32	1.23	1.20	1.03	1.18	21.50
NC-R		0.44	1.22	1.22	1.17	1.13	0.96	1.11	20.30
	P	0.15	0.06	0.07	0.13	0.08	0.12	0.12	0.10

[a] Each value is the mean of five CO_2 concentrations, two trials and two replicates within trials (=20).

Table 4. Effect of CO_2 enrichment on laminae area, laminae weight, petiole weight and specific leaf area (SLA Jaminae area/laminae weight) of leaves of two white clover clones infested with twospotted spider mites

Clone	CO_2 concn, μLL^{-1}		Trial 1[a]				Trial 2[a]			
			Laminae area, cm^2	Laminae wt, g	Petiole wt, g	Area/wt SLA	Laminae area, cm^2	Laminae wt, g	Petiole wt, g	Area/wt SLA
NC-S	395		641 (81)	21.1 (0.5)	16.7 (1.0)	30.3 (3.1)	635 (17)	14.6 (0.6)	13.4 (0.6)	43.6 (0.6)
	484		619 (37)	20.6 (1.3)	18.0 (0.6)	30.1 (0.1)	791 (24)	15.8 (1.1)	14.9 (1.0)	50.5 (2.1)
	570		714 (113)	23.0 (1.1)	19.0 (0.3)	31.0 (3.5)	864 (58)	18.0 (0.1)	16.6 (0.3)	48.0 (2.8)
	657		661 (16)	22.3 (0.4)	19.9 (0.1)	29.6 (1.2)	906 (47)	18.6 (1.2)	17.6 (1.3)	48.8 (0.6)
	748		635 (63)	22.2 (1.4)	18.6 (1.0)	28.7 (1.1)	994 (151)	20.0 (2.0)	19.8 (1.4)	49.5 (2.5)
		Model[b]								
		intercept	637	19	15	32	294	9	6	41
		slope	0.030	0.004	0.005	−0.004	0.984	0.016	0.018	0.012
NC-R	395		675 (12)	23.3 (0.8)	18.1 (1.4)	29.0 (0.5)	710 (32)	16.6 (0.2)	14.9 (0.7)	42.7 (1.4)
	484		765 (1)	26.0 (0.3)	22.2 (0.9)	29.4 (0.4)	825 (17)	18.4 (0.2)	17.0 (0.7)	44.9 (0.3)
	570		892 (24)	28.0 (2.1)	24.6 (1.4)	32.0 (1.5)	814	17.5	15.9	46.5
	657		869 (47)	27.7 (0.2)	23.3 (1.9)	31.4 (1.5)	879 (4)	19.0 (0.7)	19.0 (0.0)	46.3 (1.8)
	748		878 (9)	28.6 (1.3)	25.7 (0.2)	30.8 (1.1)	979 (73)	20.1 (0.1)	19.5 (0.4)	48.7 (3.4)
		Model[b]								
		intercept	492	19	12	27	443	13	11	36
		slope	0.585	0.015	0.020	0.006	0.699	0.009	0.012	0.016

[a] Each value is the mean (standard error) of four plants in two replicate chambers (=8), except for NC-R at 570 μLL^{-1} in trial 2 for which each value is the mean of four plants in one replicate chamber.
[b] Linear regression models. Estimated value = intercept + slope (CO_2 concentration).

SLA was not significant for the trials combined (Table 6).

Mite Reproduction. Numbers of all life stages generally increased with increasing CO_2 concentration in both trials for both clones (Table 5; Fig. 1). In trial 1, total motiles (larvae, nymphs, and adults for the clones combined) were 36, 83, 98, and 164% greater at 484, 570, 657, and 748 μLL^{-1}, respectively, than at 395 μLL^{-1} CO_2. The comparable values for trial 2, were 12, 48, 68, and 124%. For the trials combined, the overall CO_2 linear effect was significant for eggs, larvae, adults + nymphs, and total motiles (Table 6). Several life stages responded to CO_2 differently on NC-R than on NC-S in trial 1. For example, eggs showed less response to CO_2 on NC-R than on NC-S (Table 5), and the trial × clone and clone × CO_2 interactions were significant for the combined egg data (Table 6). Populations of adults + nymphs were greater on NC-R than on NC-S, and the clone effect was significant for the combined data (Table 6).

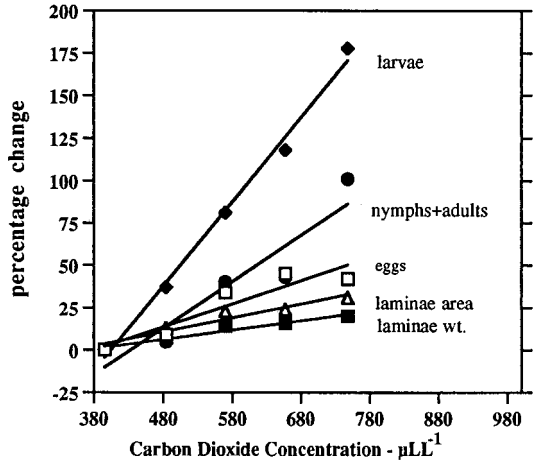

Fig. 1. Linear regressions for percentage change in mite larvae, nymphs + adults and eggs, and clover leaf area and weight. Each point is mean percentage change from 395 μLL^{-1} CO_2 as calculated for clones and trials combined using data shown in Tables 4 and 5. Regression models are: larvae = 0.497x − 201; nymphs + adults = 0.274x − 118; eggs = 0.136x − 51; laminae area = 0.083x − 29; laminae wt = 0.056x − 20.

Correlation analyses generally showed that starch and total carbohydrate concentrations were positively correlated with spider mite counts, whereas N concentrations were negatively correlated with spider mite counts (Table 7). Positive correlations with some spider mite counts occurred for some soluble carbohydrate fractions but not others, depending on the clone (Table 7). A significant negative correlation between fructans and eggs occurred for NC-R but not NC-S (Table 7).

Discussion

It is generally agreed that exposure to elevated concentrations of CO_2 and O_3 will change plant nutritive value for herbivores, and reports of changes in foliar sugars, starches, and N levels caused by elevated CO_2 and O_3 are relatively common. Although nutritive changes may be responsible for effects of CO_2 enrichment on herbivores, evidence is mostly limited to correlations between insect response and leaf analyses showing increased C, C/N ratios, and carbohydrates accompanied by decreased N (e.g., Salt et al. 1995, Brooks and Whittaker 1999, Stiling et al. 1999). Because precise nutritive requirements for success of most arthropod herbivores (including *T. urticae)* are not known, it is possible only to guess which nutritive changes may have affected reproduction in the current study. Comparison of relative effects of CO_2 and

Table 5. Effect of CO_2 enrichment on numbers of eggs, larvae, nymphs + adults, and total motiles (larvae, nymphs, and adults) of twospotted spider mites per plant on two clones of white clover[a]

Clone	CO_2 concn, μLL^{-1}		Trial 1				Trial 2			
			Eggs	Larvae	Adults + nymphs	Total motiles	Eggs	Larvae	Adults + nymphs	Total motiles
NC-S	395		2,055 (105)	57 (17)	150 (32)	207 (15)	1,870 (445)	184 (20)	184 (54)	367 (75)
	484		1,808 (229)	116 (32)	136 (14)	252 (46)	2,618 (53)	392 (168)	149 (9)	541 (159)
	570		3,297 (136)	208 (53)	179 (12)	387 (42)	3,542 (488)	450 (223)	237 (12)	687 (235)
	657		3,003 (208)	170 (59)	205 (43)	375 (102)	4,034 (869)	434 (199)	292 (3)	726 (202)
	748		2,533 (272)	181 (33)	288 (59)	468 (27)	3,550 (716)	449 (179)	293 (45)	742 (224)
		Model[b]								
		intercept	1170	−48	−32	−79	54	13	−2	10
		slope	2.40	0.34	0.39	0.73	5.37	0.65	0.41	1.05
NC-R	395		1,095 (9)	119 (6)	111 (18)	231 (12)	2,729 (464)	261 (35)	270 (54)	531 (89)
	484		1,120 (42)	136 (19)	208 (100)	344 (80)	2,916 (741)	207 (14)	259 (15)	466 (49)
	570		986 (204)	136 (54)	278 (39)	414 (15)	2,579	332	307	639
	657		1,067 (114)	209 (0)	281 (79)	490 (78)	3,100 (330)	539 (74)	243 (108)	781 (33)
	748		1,190 (45)	233 (45)	455 (44)	687 (89)	3,741 (355)	866 (158)	400 (72)	1,266 (230)
		Model[b]								
		intercept	1000	−28	−227	−252	1,575	−559	136	422
		slope	0.16	0.34	0.86	1.20	2.52	1.75	0.28	2.03

[a] Each value is the mean per plant (standared error) of four plants in two replicate chambers ($n = 8$), except for NC-R at 570 μLL^{-1} in trial 2 for which each value is the mean of four plants in one replicate chamber.
[b] Linear regression models (population estimate = intercept + slope (CO_2 concentration).

O_3 on foliar chemistry and reproduction of *T. urticae* on *T. repens* might narrow the search for nutritive factors that could be involved, however. Ozone significantly increased foliar sugars and starch in *T. repens* leaves (Burns et al. 1997), and elevated CO_2 did the same in the current study. Elevated concentrations of both gases increased *T. urticae* reproduction on *T. repens* (Heagle et al. 1994a) and elevated CO_2 did the same in the current study. However, effects of CO_2 and O_3 on foliar N concentrations were inconsistent with increased mite reproduction. For example, for an O_3-tolerant *T. repens* clone (NC-R), elevated O_3 stimulated mite reproduction but did not significantly alter foliar N (Burns et al. 1997). In the current study, there were no apparent cause–effect relationships between amino acid concentrations and mite reproduction. Possible nutrient effects were limited to the 27–29 d after infestation, and during this relatively short period, effects of increased sugars and starch could have caused the observed population increases. Conversely, any effects of decreased N would likely be more subtle, possibly requiring time for numerous generations to be detectable.

It appears unlikely that increased *T. urticae* reproduction at elevated CO_2 was related to increased leaf area. Linear regressions estimates (Fig. 1) show that for each 100 μLL^{-1} increase in CO_2, leaf area increased by 8%, whereas number of motile mites increased by 26%. Moreover, each trial ended before webbing, which signals overcrowding, was observed.

We considered the possibility that increased temperature caused the population increases we observed. Elevated CO_2 decreases stomatal conductance, thereby decreasing cooling from transpiration. Previous measures of effects of elevated CO_2 on stomatal conductance and transpiration of *T. repens* (NC-S) in our greenhouse CSTRs using a LI-COR 1600 (LI-COR) showed a small increase in leaf temperature. For example, after 14 d of 24 h d^{-1} exposure, at conditions similar to those in the current study, leaf temperature of *T. repens* (NC-S) at mid-day averaged ≈0.8°C higher at double ambient CO_2 than at ambient CO_2. Assuming no significant effect of CO_2 on stomatal conductance at night, the 24 h mean leaf temperature increase would probably be <0.4°C. A recent report (Bounfour and Tanicoshi, 2001) indicates that

Table 6. Probability levels from the analyses of variance of clover leaf and mite population response to CO_2 enrichment for the two trials combined

Source	df	Laminae area	Laminae wt	Petiole wt	Specific leaf area	Eggs	Larvae	Adults + nymphs	Total motiles
Trial	1	0.10	0.01	0.00	0.01	0.01	0.09	0.38	0.14
CO_2	4	0.14	0.01	0.01	0.51	0.10	0.15	0.02	0.01
CO_2 linear		0.02	0.00	0.00	0.17	0.02	0.03	0.00	0.00
Trial × CO_2	1	0.02	0.19	0.44	0.10	0.85	0.06	0.55	0.54
Clone	1	0.53	0.38	0.38	0.62	0.49	0.38	0.04	0.18
Trial × clone	1	0.03	0.01	0.01	0.08	0.01	0.36	0.44	0.34
Clone × CO_2	4	0.93	0.81	0.90	0.32	0.03	0.43	0.75	0.23
Trial × clone × CO_2	4	0.51	0.48	0.17	0.84	0.93	0.32	0.53	0.33

Table 7. Correlation coefficients for the relationship between spider mite populations and leaf laminae concentrations of nitrogen and nonstructural carbohydrates in two white clover clones[a]

	Nitrogen	Nonstructural carbohydrates					
		Mono-saccharides	Di-saccharides	Mono- + Disaccharides	Fructans	Starch	Total NC
NC-R clone							
Eggs	−0.42	−0.10	0.18	0.25	−0.88**	0.53	0.50
Larvae	−0.63*	−0.01	0.68**	0.27	−0.52	0.67**	0.68**
Adults + Nymphs	−0.76**	−0.32	0.54	−0.46	−0.07	0.72**	0.71**
Total Motiles	−0.76**	−0.11	0.73**	0.07	−0.44	0.77**	0.78**
NC-S clone							
Eggs	−0.68*	0.50	0.39	0.49	0.18	0.55**	0.62*
Larvae	−0.62*	0.48	−0.04	0.48	−0.23	0.54	0.55*
Adults + Nymphs	−0.84**	0.60*	0.59*	0.55*	0.21	0.80**	0.83**
Total Motiles	−0.76*	0.57*	0.16	0.55*	−0.11	0.68**	0.70**

[a] Each value is based on the mean of two replicates for the five treatments in each of two trials ($n = 10$). *, $P \leq 0.10$ and **, $P \leq 0.05$.

fecundity of *T. urticae* on red raspberry leaves was greater at 20 than at 25°C; total eggs produced per female was 125 at 20°C and 93 at 25°C. Bounifour and Tanicoshi did not measure effects of small temperature increments however, and maximum fecundity might occur at a temperature between 20 and 25°C. Therefore, it is theoretically possible that a temperature increase of <0.5°C above the mean temperatures in the present experiment (≈20°C) could affect fecundity enough to cause the population increases we observed.

We do not know why results with the "White Eye" strain of *T. urticae* on *P. vulgaris* (Joutei et al. 2000) were opposite ours. Carbon dioxide concentrations and exposure duration were similar and both studies were limited to measuring effects of exposures lasting less than two or three generations. Both studies showed increased nonstructural carbohydrates accompanied by decreased N in host leaves. Increased epidermal thickness of bean was suggested as a possible cause for decreased fecundity of the "White Eye" (Joutei et al. 2000) but we found no effect of elevated CO_2 on epidermal thickness of *T. repens* previously (Fisher et al. 1997). It is possible that differential effects of increased temperature caused by elevated CO_2 were involved. For example, if the 20°C temperature in our experiment was slightly below optimum for fecundity, a small temperature increase may have increased fecundity. Conversely, if 24°C during the experiment with "White Eye" mites was above optimum for fecundity, a small temperature increase caused by elevated CO_2 may have decreased fecundity. Identifying effects of small temperature increments on the rate of *T. urticae* population increase should be a component of future research.

The opposite results with "wild" and "White Eye" *T. urticae* preclude reasonable estimates of mite population response to elevated CO_2 concentrations expected within the next 100 yr. The results emphasize the need for research to measure effects of CO_2 enrichment over multiple mite generations on different crop species.

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